Neuroscience Module

Lecture (4)

Ammonia toxicity and encephalopathy

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Lecture Key points

- Regulation of urea cycle
- The biochemical basis of hyperammonemia

INTENDED LEARNING OBJECTIVES (ILO)

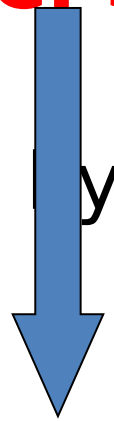
By the end of this lecture the student will be able to:

1. Categorize different methods of urea cycle regulation
2. Explain the biochemical basis of hyperammonemia
3. Determine the biochemical basis of treatment of hyperammonemia

Regulation of urea cycle

Short term regulation of urea cycle

At the level of Carbamoyl-P synthetase I (CPS I)



N Acetyl Glutamate

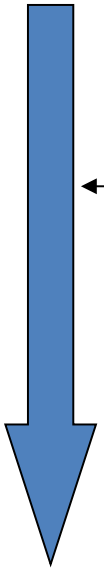
(activator of CPSI)

Synthesis & regulation of N Acetyl Glutamate

Glutamate + acetyl CoA

Arginine +

N Acetyl Glutamate Synthase



N Acetyl Glutamate+CoASH

Long term regulation of urea cycle

The enzyme levels change parallel with the protein content of diet.

By Protein –free diet

- High protein diet

- During starvation (increased protein catabolism)

Regulation of urea cycle (Quiz)

- Explain the regulation of urea cycle

Fate of urea

1) A small portion of blood urea can diffuse from the blood into the intestine, where it is cleaved by bacterial urease to CO_2 and NH_3.

NH_3 is partly reabsorbed again into the blood and partly goes to feces.

2) Excreted by the kidney.

Hyperammonemia: increased level of blood NH3

Normal serum ammonia level:

5-35 µmoL/L

Types of hyperammonemia

1) Congenital hyperammonemia :

It is due to deficiency of any enzyme of urea cycle

Comparison between carbamoyl phosphate synthetase I and ornithine transcarbamoylase deficiencies

Carbamoyl Phosphate Synthetase	Ornithine Transcarbamoylase
↑ [NH_4^+]; hyperammonemia	↑ [NH_4^+]; hyperammonemia
Blood glutamine is increased	Blood glutamine is increased
BUN is decreased	BUN is decreased
No orotic aciduria Autosomal recessive	Orotic aciduria X-linked recessive
Cerebral edema	Cerebral edema
Lethargy, convulsions, coma, death	Lethargy, convulsions, coma, death

Kaplan USMLE-1 Biochemistry and Medical Genetics

- **The two conditions can be distinguished by an increase in orotic acid and uracil,**

which occurs in ornithine transcarbamoylase deficiency, but not in the deficiency of carbamoyl phosphate synthetase.

- **Orotic acid and uracil are intermediates in pyrimidine synthesis. This pathway is stimulated by the accumulation of carbamoyl phosphate, the substrate for ornithine**

2) Acquired hyperammonemia

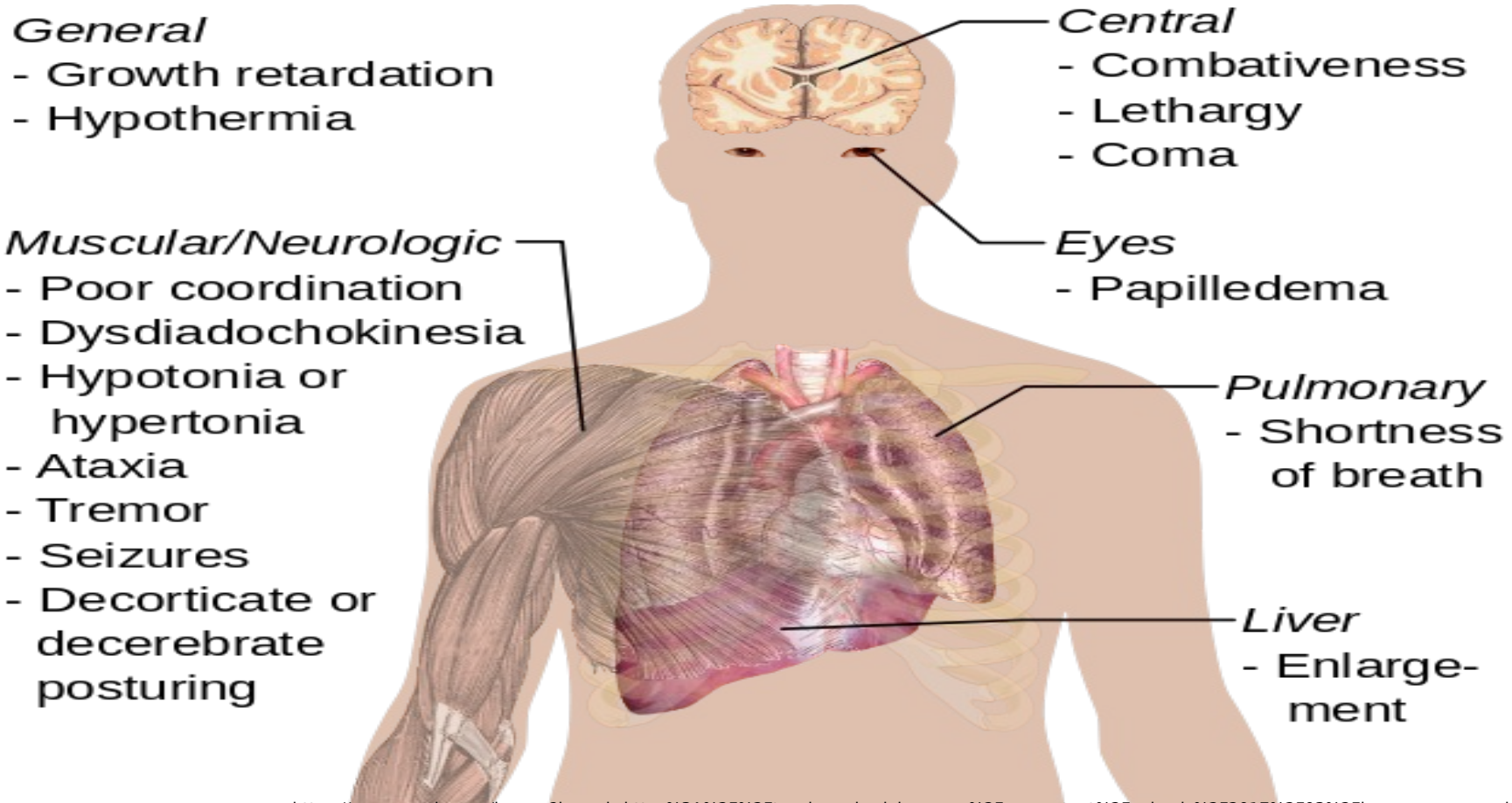
1) Liver cirrhosis

2) Hepatitis

3) Biliary obstruction

4) Alcoholism

The symptoms of hyperammonemia



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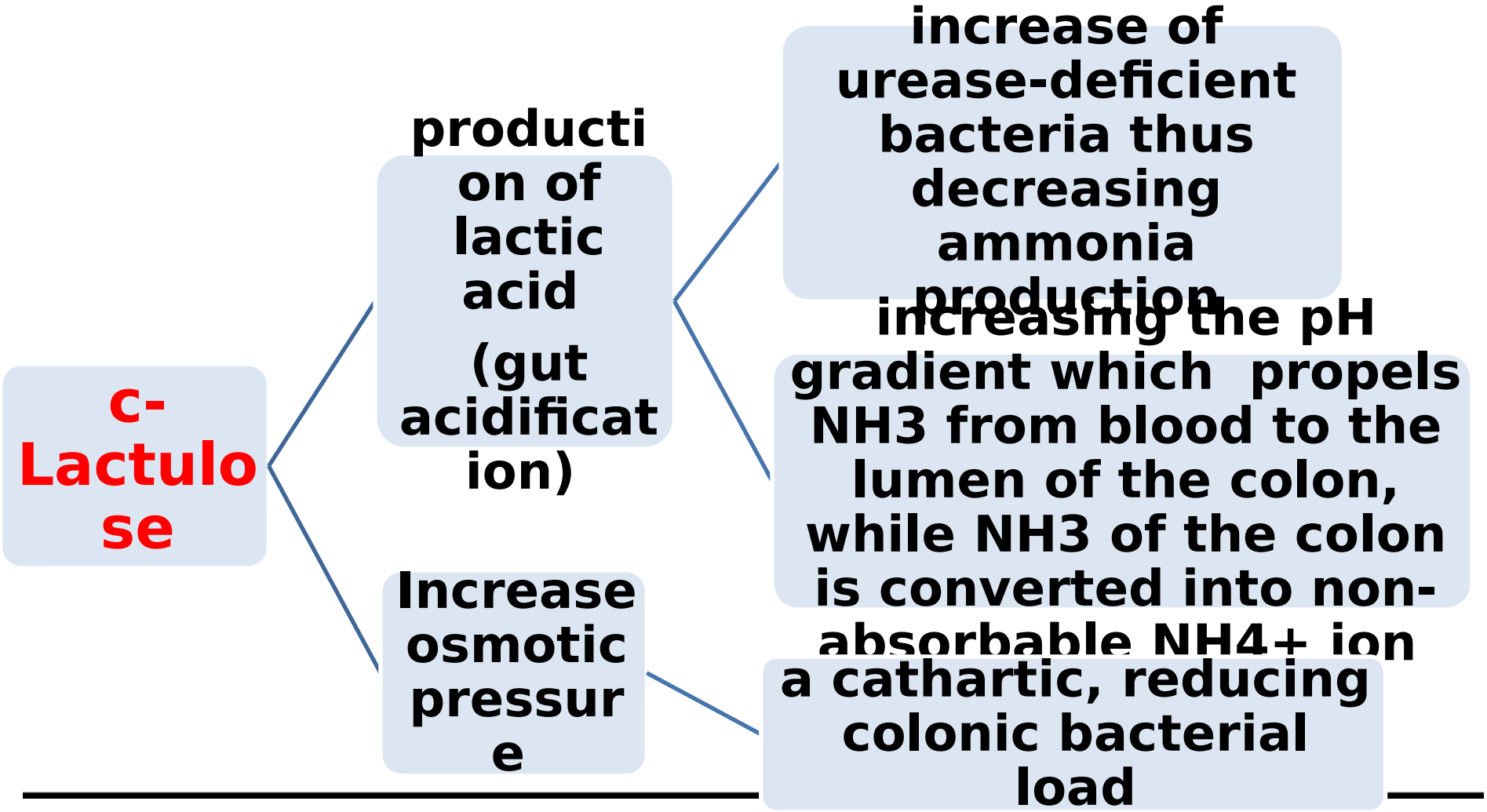
Treatment

1) Restriction of dietary protein
2) Increase carbohydrate diet to avoid proteolysis of muscle proteins
3) Decrease nitrogenous load from GIT:

a) Antibiotics (e.g neomycin) to kill intestinal bacteria

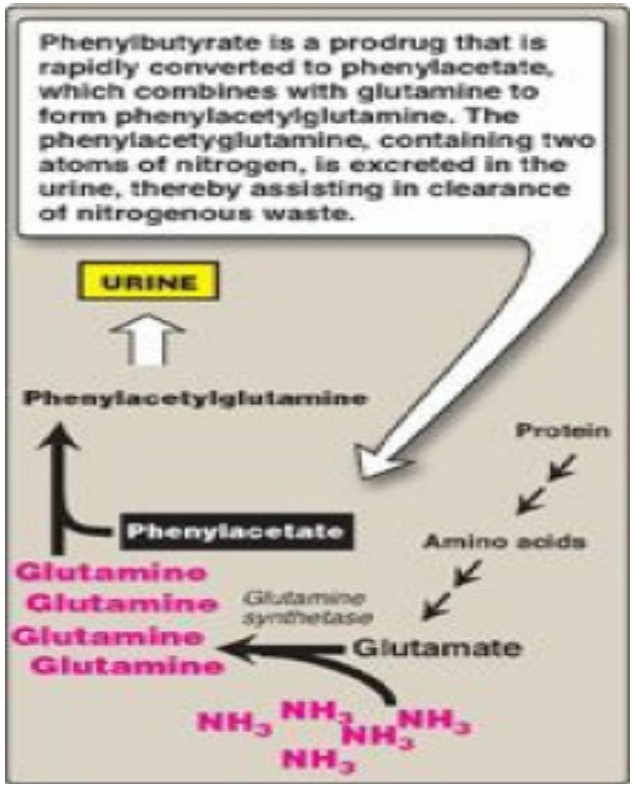
b) Enema to eliminate intestinal bacteria

Decrease nitrogenous load from GIT (continued):



The role of Phenylacetate

(Phenylbutyrate is given orally and is converted to phenylacetate which condenses with glutamine



Lippincott's illustrated reviews in Biochemistry

The role of L-ornithine-L-aspartate (LOLA, Hepa-Merz) for the brain

1) • Hepa-Merz is a combination of the amino acids ornithine and aspartate.

2) • L-ornithine stimulates the urea cycle, with resulting loss of ammonia.

3) • Both L-ornithine and L-aspartate are substrates for glutamate transaminase.

4) • Resulting in increase glutamate levels.

5) • Ammonia is subsequently utilized in the conversion of glutamate to glutamine by glutamine synthetase

The biochemical basis of hyperammonemia (Quiz)

USMLE Question

A 58-year-old man is brought by his son to the emergency department. The son reports his father as being confused, somnolent, and agitated lately. The patient also easily forgets things. The patient has a long-term history of alcoholism. He was complaining of constipation in the previous several weeks. On examination, the patient is found to be lethargic and disoriented with respect to space and time. Mental status testing shows impaired short-term memory and concentration. Bilateral asterixis and incoordination are also found. The EEG pattern is slightly abnormal. Which of the following is most likely involved in the pathophysiology of CNS changes in this patient?

- **A.** Ammonia
- **B.** Argininosuccinate synthase
- **C.** Creatinine
- **D.** Lactulose
- **E.** Sodium benzoate

EXPLANATION

The correct answer is A. Hepatic encephalopathy is a reversible metabolic encephalopathy with global CNS depression that occurs as a result of failure of the liver to detoxify toxins that escape from the intestine. It is characterized by neuropsychiatric manifestations, from lightly altered mental status to coma. Neuromuscular symptoms may be present. 40% of ammonia is generated in the intestine from ingested nitrogenous substances that are broken down by bacterial urease and amino acid oxidases. The remaining 60% is derived from the metabolism of glutamine and the deamination and transamination of other amino acids. Ammonia liberated in the intestine normally is metabolized in the liver through the Krebs-Henseleit cycle of urea synthesis into urea, which is excreted through the kidneys and into the colon. Formation of glutamine from glutamate by glutamine synthetase in the liver and brain is another means of detoxifying ammonia. Normal skeletal muscle aids in the metabolism of ammonia in the conversion of glutamate to glutamine. The muscle wasting observed in cirrhosis patients may potentiate hyperammonemia. Ammonia inhibits both excitatory and inhibitory postsynaptic potentials, thereby disturbing overall CNS function. Excess ammonia may cause cerebral energy failure due to inhibition of key rate-limiting TCA enzymes. Finally, ammonia may facilitate brain uptake of tryptophan, a substrate that generates neuroactive metabolites such as serotonin. Only nonionized ammonia crosses the membranes.

Arginosuccinate synthase (AS) **(choice B)** is a urea cycle enzyme that catalyzes the penultimate step in arginine biosynthesis—the ATP-dependent ligation of citruline to aspartate to form arginosuccinate, AMP, and pyrophosphate. In humans, a defect in the AS gene causes citrulinemia, a genetic disease characterized by severe vomiting spells and mental retardation.

Skeletal muscle contains approximately 95% of total creatine **(choice C)** pool. It is found in its free and phosphorylated form, phosphocreatine, an important muscle store of energy used for ATP synthesis from ADP. Creatine is metabolized to creatinine via a nonreversible, nonenzymatic process. Creatinine is produced at a steady rate and is affected very little by diet or by normal physical activity. Serum creatinine concentration is widely used as an index of renal function.

Lactulose **(choice D)** is a nonabsorbable disaccharide. It is thought to inhibit intestinal ammonia production by several mechanisms. It is converted by colonic bacteria into lactic acid, which results in the decrease of intestinal lumen pH. This stimulates conversion of NH_4^+ to NH_3 and facilitates the transport of NH_3 into the gut. Lactulose also acts as a cathartic, reducing ammoniagenic coliform bacteria activity. Because of above-mentioned features, lactulose is sometimes used in the management of hepatic encephalopathy.

Sodium benzoate **(choice E)** reduces serum ammonia levels by increasing ammonia excretion in urine. The mechanism involves reaction with glycine to form hippurate. The subsequent renal excretion of hippurate results in the loss of ammonia. For each mole of benzoate, the kidney excretes 1 mole of nitrogen.

Summary

- Urea cycle is subjected to both short and long term regulation
- A combination of hyperammonemia, elevated blood glutamine, and decreased blood urea nitrogen (BUN) suggests a defect in the urea cycle.
- The deficiencies of the two mitochondrial enzymes in the urea cycle, carbamoyl phosphate synthetase and ornithine transcarbamoylase can be distinguished by an increase in orotic acid and uracil, which occurs in ornithine transcarbamoylase deficiency, but not in the deficiency of carbamoyl phosphate synthetase.
- Hyperammonemia can be treated with a low protein diet and administration of drugs that provide an alternative route for capturing and excreting excess nitrogen

SUGGESTED TEXTBOOKS

- Lippincott's illustrated reviews in Biochemistry by P.C. Champe, R.A. Harvey and D.R. Ferrier
- Fundamentals of Clinical Chemistry (Tietz)
- "Textbook of Biochemistry with Clinical Correlations" by T.M. Devlin
- "Harper's Biochemistry" by R.K. Murray, D.K. Granner, P.A. Mayes and V.W. Rodwell


THANK YOU